

Comparative Pharmacokinetics of Ceftazidime in Fibrin Clots and Cardiac Vegetations in Rabbits with *Staphylococcus aureus* Endocarditis

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The penetration of ceftazidime, administered in a dose of 100 mg/kg intramuscularly, into cardiac vegetations and subcutaneously implanted fibrin clots was compared in rabbits with experimental *Staphylococcus aureus* endocarditis. Significant pharmacokinetic differences between the time-concentration curves for the two compartments were observed. Concentrations of ceftazidime in vegetations peaked at 30 min after dosing at a level slightly lower than that in plasma and thereafter declined in parallel with concentrations in plasma throughout the 8-h sampling period. Concentrations in fibrin clots increased more slowly than those in plasma and vegetations, reaching a maximum at 120 min. This was followed by a slow elimination phase yielding concentrations in excess of concurrent plasma and vegetation levels and a greater area under the curve. These features were observed for both large (2-ml volume) and small (0.1-ml volume) clots. Contrary to previous reports, these observations suggest that fibrin clots do not provide an accurate model for predicting antibiotic concentrations in cardiac vegetations produced in endocarditis and that concentrations of antimicrobial agents in vegetations can be predicted more accurately from concomitant plasma levels.

Fibrin clots have often been used in pharmacokinetic investigations, in vivo and in vitro, as models for the extravascular penetration of antimicrobial agents, particularly with respect to the fibrin-containing cardiac vegetations produced in infective endocarditis (1-4, 10-15). It has been claimed that the supposed similar composition and avascular nature of fibrin clots to those of vegetations justifies their use as a suitable model for cardiac vegetations (11, 13, 14). Previous experimental pharmacokinetic studies in rabbits have indicated, however, that although the concentrations of antibiotics in vegetations follow a pattern and time-course similar to those in plasma (7, 8; A. A. McColm and D. M. Ryan, J. Antimicrob. Chemother., in press), concentrations in clots tend to build up slowly to a peak and to decline more slowly than those in plasma (2-4, 14). None of these studies has provided a direct comparison of antibiotic pharmacokinetics in clots and vegetations. This led us to evaluate antibiotic penetration into both compartments simultaneously in rabbits with experimental bacterial endocarditis.

All previous investigations on fibrin clots have used clots of either a 1-ml or a 2-ml volume, whereas a typical cardiac vegetation from a rabbit with experimental bacterial endocarditis is much smaller (ca. 0.05 to 0.2 ml in volume). This size difference could affect the outcome of such studies; therefore, in the current investigation, antibiotic penetration was compared in "large" (2-ml-volume) and "small" (0.1-ml-volume) fibrin clots in the same animals. The β -lactamase-stable cephalosporin, ceftazidime, which is effective against experimental *Staphylococcus aureus* endocarditis in rabbits (9) and which shows rapid, high-level penetration into cardiac vegetations (McColm and Ryan, in press), was used in these investigations.

MATERIALS AND METHODS

Production of fibrin clots. Large fibrin clots (2-ml volume) were prepared by incubating 2-ml volumes of 2% aqueous

human fibrinogen (fraction 1, type III; Sigma Chemical Co.) and 0.1 ml of bovine thrombin in 0.85% saline (100 National Institutes of Health units per ml; Sigma) in sterile, siliconized test tubes (13 by 100 mm) as described by Barza and Weinstein (3). Small clots were prepared as described above but in 1-ml plastic syringes with the tips removed. The syringes were filled in the vertical position with 1-ml volumes of fibrinogen-thrombin, and after clot formation, the syringes were chilled at -20°C for 30 min to facilitate clot removal by depression of the plunger. The resulting 1-ml-volume clots were then cut into 0.1-ml-volume sections, while still chilled, with a scalpel blade.

Production of endocarditis and fibrin clot implantation. Female New Zealand White rabbits (1.8 to 2.0 kg) were anesthetized with a combination of 0.2 ml of Hypnorm (fentanyl citrate [0.315 mg/ml] plus fluanisone [10 mg/ml]; Janssen Pharmaceutica) per kg given intramuscularly and 0.1 mg of Valium (diazepam [5 mg/ml]; Roche Products Ltd., Welwyn Garden City, England) per kg given intravenously, and the right carotid artery was cannulated as described previously (9). At 72 h after cannulation, the animals were reanesthetized, and a 3-cm horizontal incision was made in the right flank. Using blunt dissection, we produced a subcutaneous space into which four large fibrin clots and four small fibrin clots were inserted as far apart as possible, after which the incisions were closed with linen suture thread (size 16; Barbour, Lisburn, Ireland). Care was taken to ensure that bunching or touching of clots did not occur during incision closure or during the remainder of the experiment. Each rabbit was inoculated intravenously with ca. 6×10^6 CFU of *S. aureus* 853E suspended in 1 ml of distilled water. This inoculum produced infection of more than 95% of all cardiac vegetations within 24 h.

Sample collection. At 24 h after bacterial inoculation and implantation of clots, groups of 10 rabbits were injected intramuscularly with a 100-mg/kg dose of ceftazidime dissolved in 0.5% sodium bicarbonate. Two rabbits were killed with pentobarbital sodium (Sagatal; May and Baker, Ltd.,

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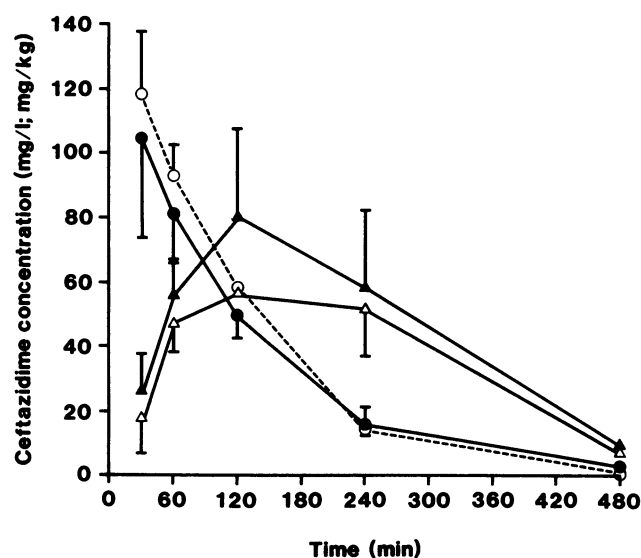


FIG. 1. Ceftazidime concentrations in plasma (○), vegetations (●), and large (2-ml-volume) (△) and small (0.1-ml-volume) (▲) fibrin clots in rabbits with experimental *S. aureus* endocarditis after an intramuscular injection of 100 mg/kg.

Dagenham, England) at 30, 60, 120, 240, and 480 min after dosing. At 10 min before sacrifice, 1,000 U of heparin in 0.85% saline was injected intravenously to prevent the adherence of blood clots to the vegetations sampled. After removal of the heart, quadruplicate samples of the cardiac vegetations and the four large and four small clots were collected, carefully blotted dry on filter paper (3), and placed into preweighed bottles which were then reweighed to five decimal places and frozen at -70°C until assayed (within 3 days). Blood samples (25- μl volume) collected in quadruplicate from the heart of each rabbit at every time point were pipetted onto thick, 6-mm-diameter Whatman assay disks. All samples were collected within 5 min of death. The experiment as described was repeated three times; the combined results produced a total of 24 replicates collected per time point for each compartment.

Antibiotic assay. Ceftazidime concentrations were measured by a large-plate agar diffusion microbiological assay with *Proteus morganii* 235 grown on diagnostic sensitivity test agar (Oxoid, Basingstoke, England). Vegetation samples were assayed whole in 4-mm-diameter miniwells sealed with molten agar (6). Because the antibiotic-containing extracellular tissue fluid contains less protein than serum does (generally about 50%), the antibiotic standards (10- μl volume) were prepared in 50% rabbit serum in phosphate-buffered saline (pH 7.0) as described by Cars and Ogren (6). Hemoglobin analysis of representative vegetations indicated that blood contamination was negligible ($\leq 3\%$). Although vegetation weights varied from ca. 10 to 50 mg in this study (mean, 34 ± 12 mg), different-sized samples removed from the same animal contained similar ceftazidime concentrations per unit weight. It was convenient in the experiments reported here to calculate concentrations of ceftazidime in plasma from the levels in whole blood as described elsewhere (McColm and Ryan, in press) by correcting for the packed-cell volume (PCV) with the following formula: $[C]_{\text{plasma}} = [C]_{\text{blood}} + \{[\text{PCV}/(100 - \text{PCV})] \times [C]_{\text{blood}}\}$. Previous studies performed in our laboratory have shown an excellent correlation between levels of ceftazidime in plasma

measured directly and those derived with the formula (correlation coefficient over the range of 0.8 to 50 mg/liter, 0.9968). A standard PCV value of 45% was used for calculating all plasma concentrations because random sampling of a large number of rabbits with endocarditis had indicated that the PCV ranged from 44 to 46% (mean \pm standard deviation, 44.8 ± 0.6).

Fibrin clots were digested for 30 min at 37°C in 0.4% bovine trypsin (type III), which was added in a 1:1 (wt/vol) ratio (12). The resulting fluid was assayed in 25- μl aliquots on four replicate 6-mm-diameter Whatman assay disks with standards prepared in 50% serum-phosphate-buffered saline. This procedure was quicker and easier than using trypsinized clots and produced identical results.

RESULTS

Ceftazidime concentrations attained in plasma, cardiac vegetations, and small (0.1-ml-volume) and large (2.0-ml-volume) fibrin clots in rabbits after an intramuscular injection of 100 mg/kg are shown in Fig. 1. Highly significant differences were seen between the antibiotic concentration-time curves for vegetations and those for fibrin clots. Ceftazidime concentrations in vegetations followed a time course similar to that for plasma, peaking at ca. 100 mg/liter within 30 min after dosing and declining steadily over the sampling period. In contrast, concentrations of ceftazidime in fibrin clots peaked 120 min after dosing to an average of 56 to 80 mg/liter (Table 1) and declined slowly thereafter. Although small clots showed higher ceftazidime concentrations than large clots at all time points, the differences were not statistically significant. There were also no significant differences between the half-lives and areas under the curve (AUC) for small and large clots (Table 1). The peak concentration of ceftazidime in vegetations was significantly higher than that in large clots ($P < 0.001$), but there was no significant difference between peak levels in vegetations and small clots (Table 1).

The half-lives of ceftazidime in clots (mean values, 71 and 76 min) were significantly longer than those in vegetations (60 ± 3 min) and plasma (54 ± 3 min) ($P < 0.001$). However, the half-life of the antibiotic in plasma did not differ significantly from that in vegetations. The AUC for large and small clots were both significantly greater than the AUC for vegetations; however, only the small clots showed a significant increase relative to plasma (Table 1).

TABLE 1. Pharmacokinetic parameters of ceftazidime in plasma, cardiac vegetations, and fibrin clots after an intramuscular injection of 100 mg/kg

Compartment	Peak concn (mg/liter; mg/kg)	Time of peak concn (min)	Half-life (min)	AUC (mg · h/liter; mg · h/kg)
Plasma	118.2 ± 19.3	30	54 ± 3	269.0 ± 29.4
Vegetations	104.2 ± 30.2^a	30	60 ± 3	236.3 ± 45.1
Small fibrin clots (0.1 ml)	80.0 ± 27.7	120	76 ± 4^b	366.4 ± 56.4^c
Large fibrin clots (2.0 ml)	56.3 ± 16.8^d	120	71 ± 4^b	297.4 ± 25.2^d

^a $P < 0.01$ relative to large clots.

^b $P < 0.001$ relative to plasma and vegetations.

^c $P < 0.05$ relative to plasma and vegetations.

^d $P < 0.05$ relative to vegetations.

DISCUSSION

The capacity of an antimicrobial agent to penetrate the infected vegetations in endocarditis is of major importance in the cure of this disease and in the speed with which cure is achieved. However, only a few experimental studies have measured directly antimicrobial penetration of cardiac vegetations in rabbits, and these have shown that the β -lactams methicillin, cefuroxime, and ceftazidime each penetrate vegetations rapidly and attain concentrations which parallel those in serum (7, 8; McColm and Ryan, in press). Previously, evidence for antimicrobial penetration of vegetations has often been extrapolated from pharmacokinetic studies with subcutaneously implanted fibrin clots in rabbits in which it was shown that antibiotics penetrate clots readily but attain lower peak levels than in plasma and have a more prolonged buildup to peak concentrations (2-4, 14).

The present paper compares the penetration of ceftazidime into infected vegetations and sterile clots in the same animals. These experiments were performed in rabbits with *S. aureus*-infected vegetations as opposed to sterile vegetations, as it is possible that tissue penetration by antibiotics may be influenced by the presence of disease. Also, infected vegetations are larger than noninfected vegetations, thus permitting easier sampling. Noninfected fibrin clots were used to conform with previous experiments (2-4, 14).

The results show that the pharmacokinetic behavior of ceftazidime penetration into fibrin clots differs greatly from that into both vegetations and serum and is characterized by a lower peak concentration, longer half-life, and greater AUC. Clot volume did not appear to be related to antibiotic penetration, as the penetration of ceftazidime into small clots (0.1-ml volume) similar in size to an average vegetation was similar to that into large clots (2-ml volume). However, the relative surface area/volume ratios of clots and vegetations are likely to influence antibiotic penetration. An ultrastructural examination of rabbit cardiac vegetations (A. A. McColm and D. J. P. Ferguson, manuscript in preparation) has confirmed their similarity to human vegetations, i.e., they are avascular, amorphous masses composed primarily of fibrin and platelets with, consequently, an extremely high surface area/volume ratio. A fibrin clot, conversely, is a relatively smooth-surfaced, homogenous compartment with a low surface area/volume ratio (13 for small clots and 4 for large clots), which may help to explain why ceftazidime penetrates more slowly into clots than into vegetations. It is likely, also, that the intravascular situation of vegetations in contact with constant arterial blood flow compared with the subcutaneous location of fibrin clots may also influence antibiotic penetration. Protein binding of ceftazidime to rabbit serum is low (ca. 35%) and therefore unlikely to influence penetration.

On the basis, therefore, of the above observations, we suggest that fibrin clots do not constitute an accurate pharmacokinetic model for studying antibiotic penetration into cardiac vegetations in endocarditis. Antibiotic concentrations in vegetations can be predicted more accurately from

the measurement of simultaneous serum concentrations (7, 8; McColm and Ryan, in press).

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